Nota Lepi. **47** 2024: 105–111 | DOI 10.3897/nl.47.118947 Short Communication

An attempt of interspecies mating between *Phengaris nausithous* and *P. teleius* (Lepidoptera, Lycaenidae)

Anna Pilařová[1], Stanislava Zachardová[1], Zdeněk Faltýnek Fric[1,2], Vladimír Vrabec[1]

1 *Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Kamýcká 129, CZ-165 00, Praha 6 – Suchdol, Czech Republic; e-mail: pilarovaa@af.czu.cz; zachardova@kam.czu.cz; vrabec@af.czu.cz*

2 *Biology Centre CAS, Branišovská 31, České Budějovice, Czech Republic; e-mail: fric@entu.cas.cz* (*corresponding author*)

https://zoobank.org/3BE80A8B-34F3-4100-97E7-B2A166B2FEA6

Received 17 January 2024; accepted 6 March 2024; published: 7 May 2024
Subject Editor: Martin Wiemers.

Abstract. Interspecies mating is rarely observed even in the wild. Such a case in Lycaenidae is reported and photographically documented for the species pairing *Phengaris teleius* (Bergsträsser, 1779) and *P. nausithous* (Bergsträsser, 1779), which was observed on 19 July 2016 in northern Bohemia near the village of Hynčice, Czech Republic. Possible causes of this phenomenon are discussed.

Introduction

From time to time, a mating between different species of butterflies is observed. The observation of such behaviour or the occurrence of hybrids in the wild is more common in some groups of butterflies than in others. More such information from the wild is available for Papilionidae (genera *Ornithoptera* Boisduval, 1832, *Papilio* Linnaeus, 1758, *Troides* Hübner, 1819) (Sands and Sawyer 1977, Sperling 2011 etc.), Pieridae (genus *Colias* Fabricius, 1807) (for instance Hovanitz 1949; Taylor 1972), Nymphalidae (genera *Danaus* Kluk, 1802, *Erebia* Dalman, 1816, *Heliconius* Kluk, 1780, *Limenitis* Fabricius, 1807) (Platt et al. 1978; Baidya et al. 2018 etc.), etc. However, relatively few observations are available for the family Lycaenidae (for instance Downey 1962; Smetacek and Bhakare 2010). The genus *Phengaris* Doherty, 1891 belonging to this family is the subject of very intensive research due to its complex ecological relationship with ants, yet we are not aware of any similar behaviour being recorded in closely related species of this genus. In the following text, we provide first information and photographic documentation about the interspecific copulation of the sympatric species *Phengaris teleius* (Bergsträsser, 1799) and *P. nausithous* (Bergsträsser, 1799), which has been observed in the wild.

Material and methods

Survey of species of the genus *Phengaris* is mandatory for the conservation of these butterflies protected by the EU Habitat Directive. Our survey of Large Blues of the genus *Phengaris* took place in the area of the Protected Landscape Area Broumovsko (Northern Bohemia, Czech

Copyright *Pilařová et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Republic) in 13 habitat patches in the vicinity of the villages of Hynčice, Ruprechtice and Vižňov which contained the flowering food plant *Sanguisorba officinalis* L. from 4 July to 5 August, 2016. The location of the patches is shown in Fig. 1. We monitored the species in this northwestern part of Czech Republic to estimate their density using the Mark-Release-Recapture method, calculated by program MARK 8.1 (White and Burnham 1999) with modifications according to Nowicki et al. (2005). Colonies of *Phengaris* butterflies here form a single metapopulation structure with dispersal occurring between patches. The total number of individuals of *Phengaris teleius* for this metapopulation was estimated by the Cormack-Jolly-Seber method (see Schwarz and Arnason 1996; Schwarz and Seber 1999) to be 802 individuals while 1487 individuals were estimated for *P. nausithous*.

We randomly took photographs of individuals throughout the survey, and the sequence of images from the interspecific mating of the aforementioned taxa was revealed later after a thorough review of the photos taken at site Hynčice 2 near the river Stěnava (GPS coordinates roughly in the middle of the territory: 50°36'58"N, 16°18'35"E, elevation about 400 m). The date of the pictures is July 19, 2016, time 13:25 CEST, by one of us (VV).

Results

After the survey in Broumovsko had finished, we encountered an unusual attempt at interspecific mating of a female *P. nausithous* and male *P. teleius* during analyses of photo-documentation (Figs 2, 3). At first, we thought it was an interesting photo of both species on a single inflorescence of a food plant, but on closer inspection, we found that the butterflies were attempting to copulate with a visible connection of their abdomens. We thus do not know the exact period of time that they remained in copula.

Figure 1. Distribution of patches where the species *Phengaris teleius* and *P. nausithous* were marked in Broumovsko in northern Bohemia of the Czech Republic in 2016. The place of observation is patch H2. Drawn in publicly available document from www.mapy.cz.

Figure 2. Individuals of *Phengaris teleius* and *P. nausithous* on a flower of either's food plant, *Sanguisorba officinalis*, while attempting to mate with each other.

Figure 3. The mutual connection of individuals of different species in this poorer quality image is evident.

Discussion

Despite these two species being closely related (see Fric et al. 2007) and that they utilise the same host plant (in Europe exclusively *Sanguisorba officinalis*), we are not aware of any other similar records of these species mating.

The vast majority of interspecific copulation cases happen within one genus. For instance, in American *Limenitis archippus* (Cramer, 1776) and related species (see Platt et al. 1978 but also Porter 1989), in the genus *Colias* (Hovanitz 1949; Taylor 1972), in *Danaus* (Baidya et al. 2018), in *Heliconius* (Mallet et al. 2007), while the best known butterfly examples are in the genus *Papilio* (Sperling 2011). Rarer are cases of mating between different genera in Lepidoptera (examples: Clarke et al. 1996; Sands and Sawyer 1977; Spitzer et al. 2010) or even between two families as in Novotný et al. (2009) between *Zygaena filipendulae* (Linnaeus, 1758) (Zygaenidae) and *Amata phegea* (Linnaeus, 1758) (Erebidae, Arctiinae) or between a butterfly (*Melitaea athalia* (Rottemburg, 1775), Nymphalidae) and a moth (*Amata phegea*) (Wiemers 1987, who also reviews some other examples).

Such intraspecific mating is a prerequisite for hybridization (Descimon and Mallet 2009); however, natural hybridization in Lepidoptera is rare. A famous example is "*Erebia serotina* Descimon & de Lesse, 1953", which is a natural hybrid of males of *E. pronoe* (Esper ,1780) and females of *E. epiphron* (Knoch, 1783) (Michel et al. 2013). The majority of known hybrids are not natural as they are produced in captivity (Wang and Dong 2001), especially between various species of the genus *Papilio* (for instance Clarke and Sheppard 1955), where even after several generations the hybrids are still fertile (Blanchard and Descimon 1988). Using molecular methods, it has been repeatedly shown that the reproductive barriers in Lepidoptera usually work well (Porter 1989; Elzinga et al. 2014). When natural interspecific mating does occur, it has been found that the willingness is strongly asymmetric between species. For instance, Deering and Scriber (2002) observed high preferences of males of *Papilio canadensis* (Rothschild & Jordan, 1906) for females of *Papilio glaucus* Linnaeus, 1758.

Observations of interspecific mating between different members of the family Lycaenidae are reported by Downey (1962) between two species of *Callophrys* Billberg, 1820 and between two species of *Lycaena* Fabricius, 1807. Further lycaenid examples are from Smetacek and Bhakare (2010), who reported mating between *Castalius rosimon* (Fabricius, 1775) and *Talicada nyseus* (Guerin, 1843) and between *Chilades parrhasius* (Fabricius 1793) and *Zizina otis* (Fabricius, 1787). For the genus *Phengaris* Doherty, 1891, however, we have not found any record of such an observation or information about the presence of hybrids anywhere, and therefore, due to the very frequent sympatry and syntopy of both species, we consider this very interesting.

What are the reasons for inter-specific mating, or even hybridisation? In general, it results from an imperfect reproduction barrier. Such barriers in Lepidoptera usually consist of several levels, many species combining olfactory recognition (Li et al. 2017) with specific behaviour and pheromones, and other signals (Phelan and Baker 1990). However, mating pheromones have frequently similar composition (Ganyard and Brady 1971, 1972) and their content vary not only between species but also within (De Pasqual et al. 2021). Also, the lock-and-key mechanism in lepidopteran genitalia does not always work (see Mikkola 1992, 2008).

Hybridization can have negative impacts when some species newly colonise or are introduced into a landscape with closely related species (Cianchi et al. 2003), but there are cases where it may

trigger new speciation (Mavarez et al. 2006; Capblancq et al. 2015). In the case of our observation, a union of butterflies was identified in the photographs, but it is far from certain whether the copulation was successful and whether additional isolation mechanisms prevented the possibility of hybrid offspring.

We cannot be sure why the observed attempted pairing of *Phengaris* butterflies occurred. As the species in Phengaris vary in number of androconia (see for instance Sibatani et al. 1994), a possible explanation is the confusion of individuals by mixing the pheromones of both species when there is a particularly high density of individuals at the observation point. Alternatively, a suitable conspecific partner might be harder to find if a pheromone is similar. However, neither reason appears particularly likely given that the population density of either species at the observation site was not higher than average, but neither was it at the limit of observability.

Acknowledgments

This work was partially supported by the Czech Ministry of Interior project VK01010103 (B.C.B.). Experiments and observations comply with the current laws of the Czech Republic where they were performed. The authors would like to thank the company HBH Projekt spol. s r. o. for financial support of field research. They also thank the administration of the Broumovsko Protected Landscape Area for the granted exemption from the protection conditions of the species of the genera *Phengaris* and *Formica*, no. 01275/BR/16. Special thanks to students of the Czech University of Life Sciences Kateřina Hejretová, Anežka Štěpánková, and Šárka Valachová for their help in the field. David Lees helped improve the English.

References

Baidya S, Basu DN, Roy S, Basu Roy A (2018) Occurrence of interspecific mating between two species of *Danaus* Kluk, 1780 (Lepidoptera: Nymphalidae) in Nature. Psyche: A Journal of Entomology 2018: e3059017. https://doi.org/10.1155/2018/3059017

Blanchard R, Descimon H (1988) Hybridization between two species of Swallowtails: meiosis mechanism, and the genesis of gynandromorphs. Journal of the Lepidopterists' Society 42: 94–102.

Capblancq T, Després L, Rioux D, Mavárez J (2015) Hybridization promotes speciation in *Coenonympha* butterflies. Molecular Ecology 24(24): 6209–6222. https://doi.org/10.1111/mec.13479

Cianchi R, Ungaro A, Marini M, Bullini L (2003) Differential patterns of hybridization and introgression between the swallowtails *Papilio machaon* and *P. hospiton* from Sardinia and Corsica islands (Lepidoptera, Papilionidae). Molecular Ecology 12(6): 1461–1471. https://doi.org/10.1046/j.1365-294X.2003.01813.x

Clarke CA, Sheppard PM (1955) The breeding in captivity of the hybrid *Papilio rutulus* female x *Papilio glaucus* male. Lepidopterists News 9: 46–48.

Clarke CA, Cronin A, Francke W, Philipp P, Pickett JA, Wadhams LJ, Woodcock CM (1996) Mating attempts between the Scarlet Tiger Moth, *Callimorpha dominula* L., and the Cinnabar Moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae), involve a common sex pheromone composition. Experientia 52(6): 636–638. https://doi.org/10.1007/BF01969746

De Pasqual C, Groot AT, Mappes J, Burdfield-Steel E (2021) Evolutionary importance of intraspecific variation in sex pheromones. Trends in Ecology & Evolution 36(9): 848–859. https://doi.org/10.1016/j.tree.2021.05.005

Deering MD, Scriber MJ (2002) Field bioassays show heterospecific mating preference asymmetry between hybridizing North American *Papilio* butterfly species (Lepidoptera: Papilionidae). Journal of Ethology 20(1): 25–33. https://doi.org/10.1007/s10164-002-0050-2

Downey JC (1962) Inter-specific mating in Lycaenidae. Journal of the Lepidopterists' Society 16: 235–237.

Elzinga J, Mappes J, Kaila L (2014) Pre- and post-mating reproductive barriers drive divergence of five sympatric species of Naryciinae moths (Lepidoptera: Psychidae). Biological Journal of the Linnean Society 112(3): 584–605. https://doi.org/10.1111/bij.12281

Fric Z, Wahlberg N, Pech P, Zrzavy J (2007) Phylogeny and classification of the *Phengaris-Maculinea* clade (Lepidoptera : Lycaenidae): total evidence and phylogenetic species concepts. Systematic Entomology 32(3): 558–567. https://doi.org/10.1111/j.1365-3113.2007.00387.x

Ganyard Jr MC, Brady UE (1971) Inhibition of attraction and cross-attraction by interspecific sex pheromone communication in Lepidoptera. Nature 234(5329): 415–416. https://doi.org/10.1038/234415a0

Ganyard Jr MC, Brady UE (1972) Interspecific Attraction in Lepidoptera in the Field. Annals of the Entomological Society of America 65(6): 1279–1282. https://doi.org/10.1093/aesa/65.6.1279

Hovanitz W (1949) Interspecific matings between *Colias eurytheme* and *Colias philodice* in wild populations. Evolution 3(2): 170–173. https://doi.org/10.2307/2405551

Li C, Wang H, Chen X, Yao J, Shi L, Zhou C (2017) Role of visual and olfactory cues in sex recognition in butterfly *Cethosia cyane cyane*. Scientific Reports 7(1): 5033. https://doi.org/10.1038/s41598-017-04721-6

Mallet J, Beltrán M, Neukirchen W, Linares M (2007) Natural hybridization in heliconiine butterflies: the species boundary as a continuum. BMC Evolutionary Biology 2007, 7: 28. https://doi.org/10.1186/1471-2148-7-28

Mavarez J, Salazar C, Bermingham E, Salcedo C, Jiggins C, Linares M (2006) Speciation by hybridization in *Heliconius* butterflies. Nature 441: 868–871. https://doi.org/10.1038/nature04738

Michel F, Meglécz E, Martin JF, Descimon H (2013) *Erebia serotina* Descimon & de Lesse 1953 (Lepidoptera: Nymphalidae), a recurrent hybrid between two distantly related species. Annales de la Société entomologique de France (N.S.) 49: 100–116. https://doi.org/10.1080/00379271.2013.774741

Mikkola K (1992) Evidence for lock-and-key mechanisms in the internal genitalia of the *Apamea* moths (Lepidoptera, Noctuidae). Systematic Entomology 17(2): 145–153. https://doi.org/10.1111/j.1365-3113.1992.tb00327.x

Mikkola K (2008) The lock-and-key mechanisms of the internal genitalia of the Noctuidae (Lepidoptera): How are they selected for? European Journal of Entomology 105(1): 13–25. https://doi.org/10.14411/eje.2008.002

Novotný D, Beneš J, Konvička M (2009) Repeated observation of interfamilial "mating" between males of the arctiid moth *Amata phegea* and females of the burnet moth *Zygaena filipendulae* (Lepidoptera: Arctiidae and Zygaenidae). Nachrichten des Entomologischen Vereins Apollo 30: 81–82.

Nowicki P, Richter A, Glinka U, Holzschuh A, Toelke U, Henle K, Woyciechowski M, Settele J (2005) Less input same output: simplified approach for population size assessment in Lepidoptera. Population Ecology 47: 203–212. https://doi.org/10.1007/s10144-005-0223-2

Phelan PL, Baker TC (1990) Comparative study of courtship in twelve phycitine moths (Lepidoptera: Pyralidae). Journal of Insect Behavior 3(3): 303–326. https://doi.org/10.1007/BF01052112

Platt AP, Rawson GW, Balogh G (1978) Inter-specific hybridization involving *Limenitis archippus* and its congeneric species (Nymphalidae). Journal of the Lepidopterists' Society 32: 289–303.

Porter AH (1989) Genetic evidence for reproductive isolation between hybridizing *Limenitis* butterflies (Lepidoptera: Nymphalidae) in Southwestern New Mexico. The American Midland Naturalist 122(2): 275–280. https://doi.org/10.2307/2425913

Sands DPA, Sawyer PF (1977) An example of natural hybridization between *Troides oblongomaculatus papuensis* Wallace and *Ornithoptera priamus poseidon* Doubleday (Lepidoptera: Papilionidae). Australian Journal of Entomology 16: 81–82. https://doi.org/10.1111/j.1440-6055.1977.tb00066.x

Schwarz CJ, Arnason AN (1996) A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics 52: 860–873. https://doi.org/10.2307/2533048

Schwarz CJ, Seber GAF (1999) Estimating animal abundance: Review III. Statistical Science 14: 427–456. https://doi.org/10.1214/ss/1009212521

Sibatani A, Saigusa T, Hirowatari T (1994) The genus *Maculinea* van Eecke, 1915 (Lepidoptera: Lycaenidae) from the East Palaearctic Region. Tyo to Ga 44(4): 157–220.

Smetacek P, Bhakare S (2010) Two instances of inter-generic mating by Lycaenidae (Lepidoptera) in Maharashtra, India. Journal of Research on the Lepidoptera 43: 23–25. https://doi.org/10.5962/p.266506

Sperling F (2011) Natural hybrids of *Papilio* (Insecta: Lepidoptera): poor taxonomy or interesting evolutionary problem? Canadian Journal of Zoology 68: 1790–1799. https://doi.org/10.1139/z90-260

Spitzer L, Beneš J, Vrba P, Zlatník M (2010) Tree observation of interspecific mating attempts by males of the Meadow Brown (*Maniola jurtina* (Linnaeus, 1758)) in the wild (Lepidoptera, Nymphalidae: Satyrinae, Heliconiinae). Nachrichten des Entomologischen Vereins Apollo 31(3): 166–168.

Taylor OR (1972) Random vs. non-random mating in the Sulfur butterflies, *Colias eurytheme* and *Colias philodice* (Lepidoptera: Pieridae). Evolution 26(3): 344–356. https://doi.org/10.1111/j.1558-5646.1972.tb01940.x

Wang CZA, Dong J (2001) Interspecific hybridization of *Helicoverpa armigera* and *H. assulta* (Lepidoptera: Noctuidae). Chinese Science Bulletin 46: 489–491. https://doi.org/10.1007/BF03187264

White GC, Burnham KP (1999) Program MARK: Survival estimation from populations of marked animals. Bird Study 46: 120–138. https://doi.org/10.1080/00063659909477239

Wiemers M (1987) Beitrag zur artfremden Freilandpaarung. Eine Freilandkopula zwischen *Mellicta* (Lep.: Nymphalidae) und *Amata* (Lep.: Ctenuchidae) sowie eine weitere Zygaenen-Hybridkopula (Lep.: Zygaenidae). Entomologische Zeitschrift mit Insektenbörse 97(3): 27–30.